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Amino acid sequences of nerve growth factors derived from cobra venoms

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Amino acid sequences of nerve growth factors (NGFs), purified from the venoms of Indian cobra (*Naja naja*) and Thailand cobra (*Naja naja siamensis*) were determined. The sequence of *N. naja* NGF differed from that reported previously by Hogue-Angeletti et al. [(1976) Biochemistry 15, 26–34]. The sequence of *N. naja siamensis* NGF was identical to that of Formosan cobra *Naja naja atra* NGF, determined previously by Oda et al. [(1989) Biochem. Int. 19, 909–917] and to that deduced from the nucleotide sequence of an NGF cDNA from the venom gland of *N. naja siamensis*, as reported by Selby et al. [(1987) J. Neurosci. Res., 18, 293–298].

Amino acid sequence; Nerve growth factor; *Naja naja*; *Naja naja siamensis*

1. INTRODUCTION

Nerve growth factor (NGF) is a polypeptide hormone, that is necessary for the survival both in vivo and in vitro of embryonic sympathetic and sensory neurons [1]. Though snake venom is one of the most abundant sources of NGF, studies on snake venom NGFs have not been made so frequently as those on mouse NGF. It is important to determine the sequences of various snake venom NGFs in order to explain the structure/function relationships of NGF.

Previously, we determined the amino acid sequence of NGF from the venom of the Formosan cobra, *N. naja atra* [2]. In this report, we provide the amino acid sequences of NGFs from the venoms of the Indian cobra *Naja naja* and the Thailand cobra *Naja naja siamensis*.

2. MATERIALS AND METHODS

2.1. *Isolation of NGFs*

NGFs were isolated from the venoms of *Naja naja* (Sigma) and *Naja naja siamensis* (Sigma) as described previously [2]. The homogeneity of each was confirmed by isoelectric focusing in the presence of 8 M urea [3] and SDS-polyacrylamide electrophoresis [4]. Biological activities of the purified NGFs toward chicken dorsal root ganglia were estimated by the method described by Varon et al. [5].

S-Pyridylethylated (PE) derivatives of NGFs were prepared by the method described previously [6] in the presence of 6 M guanidine hydrochloride (Nacalai Tesque, Japan).

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Abbreviations: NGF, nerve growth factor; HPLC, high-performance liquid chromatography; PE, *S*-pyridylethyl; PEC, *S*-pyridylethylcysteine; N, amino; C, carboxyl

2.2. *Studies on the amino acid sequences of NGFs*

PE-NGFs were cleaved with cyanogen bromide [7]. The peptides thus obtained were separated by reverse phase HPLC on a VYDAK 214TP54 column (The Separation Group, USA) with 0.05% trifluoroacetic acid containing a linear gradient from 0% to 60% acetonitrile. Cyanogen bromide peptide IM-2 derived from *N. naja* NGF was further cleaved with staphylococcal protease (Miles Scientific, USA) in 0.1 M Tris-HCl buffer (pH 9.0) at 37°C for 6 h (enzyme/substrate weight ratio of 1:20).

Amino acid sequences of PE-NGFs and their fragments were determined with a protein/peptide sequencer (Applied Biosystems, model 477A) equipped with an on-line phenylthiohydantoin (PTH) analyzer (model 120A).

The C-terminal sequences of PE-NGFs were determined by carboxypeptidase Y (Cooper Biomedical) digestion at 37°C for 120 min in 0.05 M Tris-HCl buffer (pH 8.0) containing 2 M urea [8]. The released amino acids were determined with an amino acid analyzer.

2.3. *Amino acid analysis*

The protein and peptide samples were hydrolyzed in 6 N HCl containing 0.2% phenol at 110°C for 24 h in evacuated sealed tubes. The amino acid compositions were determined with an amino acid analyzer (Hitachi model L-8500).

3. RESULTS

3.1. *Comparison of cobra venom NGFs*

NGFs were purified from the cobra venoms (Indian cobra *Naja naja* and Thailand cobra *Naja naja siamensis*) by Sephadex G-50 gel filtration, CM-Sephadex C-50 chromatography, Mono S chromatography, and reversed-phase HPLC. Purified preparations of these two cobra NGFs showed practically the same biological activities toward chicken dorsal ganglia; but, as previously observed [9], these NGFs showed lower activities than mouse NGF with respect to the increase in length of neurites projecting from the ganglia. From the isoelectrofocusing gel electrophoresis, the isoelectric point of *N. naja* NGF (p*I* 6.8) was found to be

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Table I

Amino acid compositions of *N. naja* and *N. naja siamensis* NGFs

Amino acid	*N. naja* NGF		*N. naja siamensis* NGF	
Asp	16.9	(17)	16.1	(16)
Thr	12.9	(14)	12.1	(13)
Ser	7.34	(8)	7.06	(8)
Glu	9.09	(9)	9.90	(10)
Pro	3.57	(4)	3.90	(4)
Gly	6.87	(6)	6.28	(6)
Ala	5.11	(5)	5.12	(5)
Val	9.51	(10)	9.58	(10)
Met	1.73	(2)	1.84	(2)
Ile	5.60	(6)	5.62	(6)
Leu	3.28	(3)	3.11	(3)
Tyr	2.69	(3)	2.86	(3)
Phe	3.86	(4)	3.95	(4)
Lys	8.81	(9)	9.84	(10)
His	3.71	(4)	3.87	(4)
Arg	3.04	(3)	2.98	(3)
PEC[a]	5.24	(6)	5.03	(6)
Trp[b]	N.D.	(3)	N.D.	(3)
Total		116		116

Values in parentheses were taken from the sequences.
[a]Cys was determined as pyridylethylcysteine (PEC)
[b]Trp was not determined (N.D.)

slightly lower than that of *N. naja siamensis* NGF (p*I* 7.2). Table I shows the amino acid compositions of these NGFs.

3.2. *Amino acid sequence of* Naja naja *NGF*

The N-terminal sequence of *N. naja* NGF was determined with the sequencer up to residue 40. The C-terminal sequence was determined to be Gly-Asn-COOH by carboxypeptidase Y digestion. PE-NGF was cleaved with cyanogen bromide, and the peptides were separated by HPLC into three fragments (IM-1 to IM-3), which contained the amino acid residues from 1 to 37, from 38 to 90, and from 91 to 116, respectively. Since IM-2 was too long to be sequenced completely, it was further cleaved with staphylococcal protease, and three peptides (IM-2-E-1 to IM-2-E-3) obtained were sequenced. On the basis of the sequences of these peptides, the *N. naja* NGF was completely sequenced and was found to be composed of 116 residues as shown in Fig. 1, giving a total molecular mass of 13015 Da.

3.3. *Amino acid sequence of* Naja naja siamensis *NGF*

The N-terminal sequence of *N. naja siamensis* NGF was determined with a sequencer up to residue 42. The C-terminal sequence, Gly-Asn-COOH, determined by carboxypeptidase Y digestion, was the same as that for the *N. naja* NGF. The three cyanogen bromide peptides (TM-1 to TM-3) were completely sequenced. Thus the PE-NGF was found to be composed of 116 amino acid residues as shown in Fig. 1, yielding a total molecular weight of 13 057 Da.

4. DISCUSSION

Fig. 1 compares the amino acid sequences of *N. naja* and *N. naja siamensis* NGFs determined in the present study, with those of *N. naja atra* and mouse NGFs [2, 10]. Previously Hogue-Angeletti et al. reported a ten-

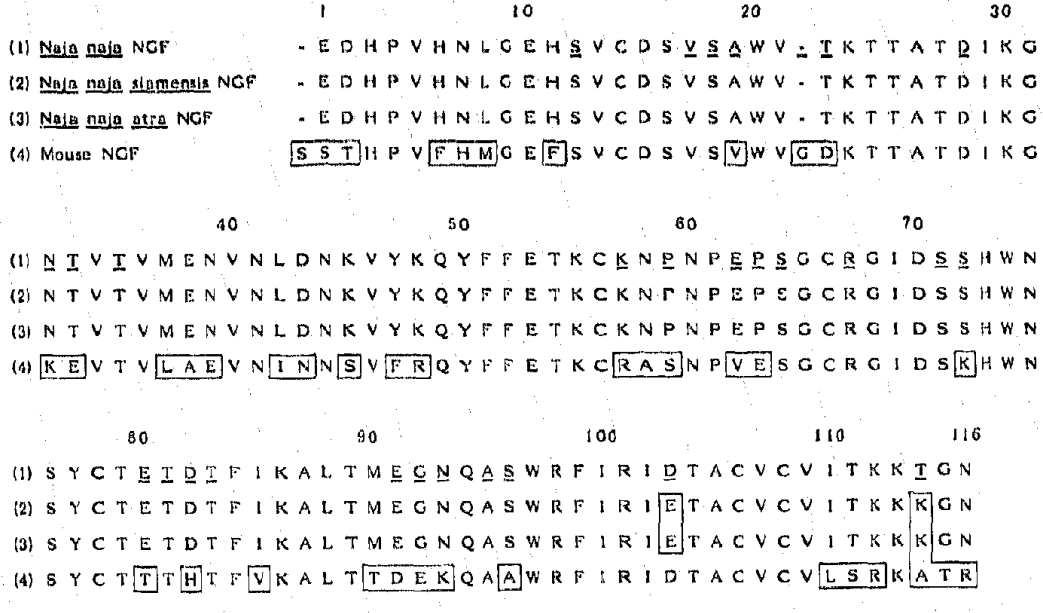

Fig. 1. Comparison of the amino acid sequences of four NGFs. NGFs were from the Indian cobra, *N. naja* (present study); the Thailand cobra, *N. naja siamensis* (present study); the Formosan cobra, *N. naja atra* [2]; and the mouse [10]. The underlined residues of *N. naja* NGF differ from those of the sequence reported by Hogue-Angeletti et al. [11]. The boxed residues indicate the amino acid residues that differ those for *N. naja* NGF.

tative sequence of *N. naja* NGF that was based on the partial sequences and amino acid compositions of the peptides [11]. The sequence determined in the present study, however, differed in 29 amino acid residues from that reported previously, and these residues are underlined in Fig. 1. The amino acid sequence of *N. naja siamensis* NGF determined in the present study was identical to that of *N. naja atra* NGF [2] and also to that deduced from the nucleotide sequence of an NGF cDNA from the venom gland of *N. naja siamensis* [12].

Two amino acid replacements were observed between the sequences of *N. naja* and *N. naja siamensis* NGFs. We determined previously the amino acid sequences of cytotoxin-like basic proteins (CLBPs) derived from the venoms of three cobras (*N. naja*, *N. naja siamensis*, and *N. naja atra*) [13,14]. In contrast to that of NGF, the sequence of *N. naja siamensis* CLBP was found to differ from the other two cobra CLBPs in two amino acid residues and the sequence of *N. naja* CLBP was identical to that of *N. naja atra* CLBP. Since the two amino acid replacements in the cobra NGFs had no effect on the biological activity, these residues are thought to be nonessential for NGF activity. As cobra NGFs show lower biological activity than mouse NGF, only a few of the 40 amino acid differences found between the sequences of cobra and mouse NGFs (Fig. 1) might be responsible for the difference in the biological activity between cobra and mouse NGFs.

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